

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED / ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER P67678US0
INTERNATIONAL APPLICATION NO PCT/NZ00/00176	INTERNATIONAL FILING DATE 7 September 2000	US APPLICATION NO (known to 37 CFR 1.55) 10/069527
TITLE OF INVENTION SEEDLESS FRUIT PRODUCTION		PRIORITY DATE CLAIMED 7 September 1999
APPLICANT(S) FOR DO/EO/US Jialong YAO and Bret MORRIS		

Applicant herein submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for Internatl. Preliminary Examination was made by the 19th month from earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the Internatl. Preliminary Examination report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
 - International Search Report
 - PCT Request Form
 - First Page of Publication
 - Demand
 - International Preliminary Examination Report - with no annexes
 - Sequence Listing

US APPLICATION NO (If known, see 37 CFR 1.51) <div style="font-size: 2em; font-weight: bold; text-align: center;">10/069527</div>		INTERNATIONAL APPLICATION NO <div style="font-weight: bold; text-align: center;">PCT/NZ00/00176</div>		ATTORNEY'S DOCKET NUMBER <div style="font-weight: bold; text-align: center;">P67678US0</div>					
17. <input checked="" type="checkbox"/> The following fees are submitted: Basic National Fee (37 CFR 1.492(a)(1)-(5)): Internatl. prelim. examination fee paid to USPTO (37 CFR 1.492 (a) (1)) .. \$710.00 No international preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (2)) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .. \$740.00 Neither international preliminary examination fee (37 CFR 1.492 (a) (3)) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO) \$1040.00 International preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (4)) and all claims satisfied provisions of PCT Article 33(2)-(4) \$100.00 Search Report prepared by the EPO or JPO (37 CFR 1.492 (a) (5)) \$890.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>				<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <th style="width:50%;">CALCULATIONS</th> <th style="width:50%;">PTO USE ONLY</th> </tr> <tr> <td style="height: 100px; vertical-align: bottom;">\$ 1040.00</td> <td></td> </tr> </table>		CALCULATIONS	PTO USE ONLY	\$ 1040.00	
CALCULATIONS	PTO USE ONLY								
\$ 1040.00									
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <td style="width:50%;">\$</td> <td style="width:50%;"></td> </tr> </table>		\$			
\$									
Claims		Number Filed	Number Extra	Rate					
Total Claims		34 - 20 =	-14-	x \$18.00	\$ 252.00				
Independent Claims		9 - 3 =	-6-	x \$84.00	\$ 504.00				
Multiple Dependent Claim(s) (if applicable)				+ \$280.00	\$				
TOTAL OF ABOVE CALCULATIONS =					\$ 1796.00				
Reduction by 1/2 for filing by small entity , if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).					\$				
SUBTOTAL =					\$ 1796.00				
Processing fee of \$130 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f))					\$				
TOTAL NATIONAL FEE =					\$ 1796.00				
Fee of \$40.00 for recording the enclosed assignment (37 CFR 1.21(h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31).					\$ 40.00				
TOTAL FEES ENCLOSED =					\$ 1836.00				
					Amt. to be refunded:	\$			
					Amt. charged:	\$			
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>1836.00</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. <u>06-1358</u> in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge my account any additional fees set forth in §1.492 during the pendency of this application, or credit any overpayment to Deposit Account No. <u>06-1358</u> . A duplicate copy of this sheet is enclosed.									
SEND ALL CORRESPONDENCE TO: <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> JACOBSON HOLMAN PLLC 400 7th Street, N.W., Suite 600 Washington, DC 20004 202-638-6666 CUSTOMER NUMBER: 00136 </div> <div style="width: 45%; text-align: right;"> By <u></u> John C. Holman Reg. No. 22,769 </div> </div>									

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Jialong YAO et al.

Serial No.: New

Filing Date: March 6, 2002

FOR: SEEDLESS FRUIT PRODUCTION

Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE SPECIFICATION

On page 1, immediately following the title, please insert the following sentence: --This is a nationalization of PCT/NZ00/00176 filed September 7, 2000 and published in English.--

IN THE CLAIMS

Please amend claims 3, 8, 11, 12, 19, 20, 25, 26, 29, 33 & 34 as follows:

3. (amended) A fruiting plant according to claim 1 which produces a pome fruit.

8. (amended) A plant as claimed in claim 4 wherein said plant is one which produces pome fruit.

11. (amended) A plant as claimed in claim 8, in which said polynucleotide (b) has the coding sequence of SEQ ID NO: 3.

12. (amended) A plant as claimed in claim 8, wherein said polynucleotide (b) has the nucleotide sequence of SEQ ID NO: 3.

19. (amended) A DNA construct which includes a polynucleotide as claimed in claim 13 .

20. (amended) A DNA construct comprising, in the 5'-3' direction:

- (a) a promoter sequence;
 - (b) an open reading frame polynucleotide as defined in claim 13; and
 - (c) a termination sequence.
25. (amended) A DNA construct as claimed in claim 23 [or claim 24] in which the non-coding region is in a sense orientation.

25. (amended) A DNA construct as claimed in claim 23 in which the non-coding region is in a sense orientation.

26. (amended) A DNA construct as claimed in claim 23 in which the non-coding region is in an anti-sense orientation.

29. (amended) A transgenic cell of a fruiting plant which includes a DNA construct as claimed in claim 19.

33. (amended) A seedless or sterile fruit which is produced by a fruiting plant as claimed in claim 1.

34. (amended) A seedless or sterile pome fruit which is produced by a fruiting plant as claimed in claim 3.

REMARKS

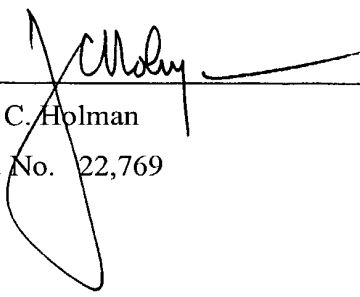
The foregoing Preliminary Amendment is requested in order to delete the multiple dependent claims and avoid paying the multiple dependent claims fee.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned “ **VERSION WITH MARKINGS TO SHOW CHANGES MADE.**”

Early action on the merits is respectfully requested.

Respectfully submitted,
JACOBSON HOLMAN PLLC

By


John C. Holman

Reg. No. 22,769

400 Seventh Street, N.W.
Washington, D.C. 20004-2201
(202) 638-6666

Atty. Docket: P67678US0
Date: March 6, 2002
JCH/cmf

VERSION WITH MARKINGS TO SHOW CHANGES MADE

3. (amended) A fruiting plant according to claim 1 [or claim 2] which produces a pome fruit.

8. (amended) A plant as claimed in claim 4 [any one of claims 4 to 7] wherein said plant is one which produces pome fruit.

11. (amended) A plant as claimed in claim 8, [claim 9 or claim 10] in which said polynucleotide (b) has the coding sequence of SEQ ID NO: 3.

12. (amended) A plant as claimed in claim 8, [claim 9 or claim 10] wherein said polynucleotide (b) has the nucleotide sequence of SEQ ID NO: 3.

19. (amended) A DNA construct which includes a polynucleotide as claimed in claim 13 [any one of claims 13 to 18].

20. (amended) A DNA construct comprising, in the 5'-3' direction:

- (a) a promoter sequence;
 - (b) an open reading frame polynucleotide as defined in claim 13 [any one of claims 13 to 18]; and
 - (c) a termination sequence.
25. (amended) A DNA construct as claimed in claim 23 [or claim 24] in which the non-coding region is in a sense orientation.

25. (amended) A DNA construct as claimed in claim 23 [or claim 24] in which the non-coding region is in a sense orientation.

26. (amended) A DNA construct as claimed in claim 23 [or claim 24] in which the non-coding region is in an anti-sense orientation.

29. (amended) A transgenic cell of a fruiting plant which includes a DNA construct as claimed in claim 19 [any one of claims 19 to 28].

33. (amended) A seedless or sterile fruit which is produced by a fruiting plant as claimed in claim 1 [any one of claims 1, 2, 4-7 and 31].

34. (amended) A seedless or sterile pome fruit which is produced by a fruiting plant as claimed in claim 3 [any one of claims 3, 8 to 12 and 32].

13 Rec'd PCT/PTO 27 JUN 2002

10/069527

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Jialong YAO et al.

Filed: March 6, 2002

Art Group: To Be Assigned

Serial No.: 10/069,527

Examiner: To Be Assigned

For: SEEDLESS FRUIT PRODUCTION

**AMENDMENT AND SUBMISSION OF SUBSTITUTE
SEQUENCE LISTING UNDER 37 C.F.R. §1.825(a)**

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

In response to the Notification of Missing Requirements Under 35 U.S.C. 371

Application Papers mailed May 10, 2002, kindly amend the captioned application as follows:

AMENDMENT

In the Sequence Listing:

Please replace the existing Sequence Listing for the above-identified application with the
Substitute Sequence Listing appended hereto.

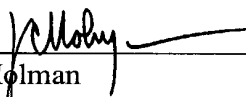
CONCLUSION

It is respectfully believed this application is now in condition for allowance. Early notice to this effect is earnestly solicited.

It is not believed that extensions of time or other are required beyond those that may otherwise be provided for herewith. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. §1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our **Deposit Account No. 06-1358, Attorney Docket No. P67678US0.**

Respectfully submitted,

JACOBSON HOLMAN, PLLC



 John C. Holman
 Reg. No. 22,769

Date: June 26, 2002

The Jenifer Building
 400 Seventh Street, N.W.
 Washington, DC 20004-2201
 (202) 638-6666

SEQUENCE LISTING

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WO 01/17334

PCT/NZ00/00176

SEEDLESS FRUIT PRODUCTION

FIELD OF THE INVENTION

- 5 The invention provides plants that produce seedless or sterile fruit.

BACKGROUND TO THE INVENTION

10 The production of seedless or parthenocarpic fruit is a desirable trait for commercially grown cultivars. Seedless fruit are more convenient than seeded fruit to consumers. Furthermore parthenocarpic fruit trees can be cropped without pollination, which reduces dependence on bees, pollinator varieties and warm weather at flowering. The absence of pollen is also advantageous so as to alleviate environmental concerns regarding the transfer of transgenes to non-transgenics by
15 cross-pollination.

Seedless fruit cultivars can also avoid or reduce biennial bearing tendencies that have been attributed to the inhibition of flower bud formation by developing seeds in apple (Chan and Cain, 1967). Seedless apple fruit is also much less susceptible
20 to codling moth, a major pest on apple trees, compared to seeded fruit (Goonewardene *et al.*, 1984).

The applicants have now identified and isolated a reproductive gene which encodes a peptide involved in the reproductive (seed-producing) cycle of fruiting plants,
25 particularly apple trees. It is broadly towards this gene, to its homologs in other fruiting plants and to the modulation of its expression/function within fruiting plants that the present invention is directed.

SUMMARY OF THE INVENTION

30

In a first aspect, the present invention provides a fruiting plant which has been genetically modified such that it does not functionally express:

- 35 (i) a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and/or

- (ii) a peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof,

5 which plant produces seedless or sterile fruit.

In a further aspect, the invention provides a fruiting plant which contains a polynucleotide encoding a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof and in which the functional
10 expression of said peptide within said plant has been disrupted such that the plant produces seedless or sterile fruit.

In still a further aspect, the invention provides a fruiting plant which contains:

- 15 (a) a polynucleotide encoding a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and
- (b) a polynucleotide encoding a peptide having the *MdAP3* amino acid
20 sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof,

and in which the functional expression of said peptide encoded by polynucleotide (a) within said plant has been disrupted such that the plant produces seedless or
25 sterile fruit.

In one form, functional expression of said peptide encoded by polynucleotide (a) is disrupted directly.

30 In another form, functional expression of said peptide encoded by polynucleotide (a) is disrupted indirectly, such as through disrupting functional expression of the peptide encoded by said polynucleotide (b).

As used herein, "fruiting plant" means a plant in which the fruit is formed from the
35 ovary and the fused bases of sepals, petals and stamen, whereas "functional

expression" of said peptide refers to the amount of the peptide which is expressed and functional within the plant. For example, a plant which does not functionally express a peptide can mean either that there is no expression of that peptide at all, or that the peptide is expressed but no longer performs its previous function.

5

Conveniently, the plant is one which produces a pome fruit.

Disruption of functional expression may be by mutation (such as frameshift, deletion, insertion or knockout mutations) of the gene itself or of its regulatory elements, down-regulation (such as antisense, co-suppression) or any other method known to those skilled in the art by which aberrant or reduced expression of the gene may be achieved (e.g. Montgomery and Fire, 1998).

10

Disruption may therefore be specifically caused by down-regulation of expression of *MdPI* by down-regulation of expression of inter-related *MdAP3*, or both.

15

In a further embodiment, the invention provides a polynucleotide which encodes a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a variant thereof, or which encodes a peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a variant thereof.

20

Most preferably, said polynucleotide includes part or all of the nucleotide sequence of SEQ ID NO: 1, or part or all of the nucleotide sequence of SEQ ID NO: 3.

25 Preferably, the polynucleotide is DNA.

The invention further provides a DNA construct which includes a polynucleotide as defined above.

30 More particularly, the invention provides a DNA construct comprising, in the 5'-3' direction:

(a) a promoter sequence;

Similar constructs can also be provided including a polynucleotide which encodes part or all of the *MdAP3* peptide having the sequence of SEQ ID NO: 4.

In still a further aspect, the invention provides a transgenic fruiting plant cell which includes a DNA construct as defined above, as well as a transgenic fruiting plant comprising such cells.

Finally, the invention includes seedless or sterile fruit produced by a plant as defined above.

10

DESCRIPTION OF THE DRAWINGS

While the invention is broadly defined as above, those persons skilled in the art will appreciate that it is not limited thereto and that it also includes embodiments of which the following description provides examples or which are the subject of specific claims. In addition, the present invention will be better understood from reference to the accompanying drawings in which:

Figure 1 shows the phenotype of wild type and Rae Ime apple flowers and fruit.

20

- (a) normal apple flowers showing sepals, petals, stamens and styles.
- (b) a normal 5-week-old apple fruit showing five carpels with 0 to 2 seeds per carpel.
- (c) Rae Ime flowers with no petals or stamens but with increased numbers of styles.
- (d) cross sections at the lower part (left) and upper part of a 5-week-old Rae Ime fruit, showing two whorls of carpels without seed.
- (e) top of Rae Ime fruit showing two whorls of calyxes.
- (f) top of normal apple fruit showing a whorl of calyxes.
- (g) mature fruit of Rae Ime with size of 5 cm wide and no seed.

30

Figure 2 shows the sequence of *MdPI*. The cDNA sequences and deduced amino acid sequences of *MdPI* isolated from Granny Smith apple are shown. Gene specific PCR primers are underlined. Primer directions are indicated with horizontal arrows. Intron positions are indicated with vertical arrows.

35

Figure 3 shows a Northern blot analysis of apple RNA sample using *MdPI* cDNA as a probe. RNA sample were prepare from ovaries (1), sepals (2), young leaves (3), skin (4), cortex (5) and core (6) tissue of 4-week-old fruit of Granny Smith, 1-week-old fruit (7), flower peduncles (8), stamens (9), petals (10) of Granny Smith (12), flower buds of Rae Ime (11), and flower buds of Granny Smith (12).

Figure 4 shows a Southern analysis of apple genomic DNA using *MdPI* cDNA as a probe. DNA of Rae Ime (Ri) and Granny Smith (Gs) were digested with EcoRI (E) and HindIII (H).

Figure 5 shows the identification of a transposon insertion in *MdPI* of Rae Ime, Spencer Seedless and Wellington Bloomless.

- (a) Genomic DNA fragments were amplified using primers P3 and P7 from Rae Ime (Ri) and Granny Smith (Gs).
- (b) Southern blot made from the gel shown in (a) was probed with the cDNA of *MdPI*.
- (c) The genomic DNA of *MdPI* from Granny Smith, Rae Ime, Spencer Seedless and Wellington Bloomless was sequenced. The sequence of *MdPI* of Granny Smith was numbered from the ATG start codon. The black boxes are the coding regions and the white box is the 3' non-coding region. A transposon insertion was found in the intron 4 of *MdPI* of Rae Ime and in the intron 6 of Spencer Seedless (Ss) and Wellington Bloomless (Wb) as shown by the arrows.

Figure 6 shows the cDNA and deduced amino acid sequences of *MdAP3*.

DESCRIPTION OF THE INVENTION

As broadly outlined above, the applicants have identified a peptide which is involved in fruiting plant reproduction, together with the gene coding therefor. The

specific peptide and gene are from a plant which produces pome fruit, *Malus x domestica*.

5 The amino acid sequence of one peptide, *MdPI*, and its encoding nucleotide sequence are given in Figure 2. It will however be appreciated that the invention is not restricted only to the peptide/polynucleotide having the specific amino acid/nucleotide sequence given in Figure 2. Instead, the invention also extends to functionally equivalent variants of the peptide/polynucleotide of Figure 2.

10 The term "polynucleotide(s)" as used herein means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA
15 molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of
20 "polynucleotide" therefore includes all such operable anti-sense fragments.

The phrase "functionally equivalent variants" recognises that it is possible to vary the amino acid/nucleotide sequence of a peptide while retaining substantially equivalent functionality. For example, a peptide can be considered a functional
25 equivalent of another peptide for a specific function if the equivalent peptide is immunologically cross-reactive with and has at least substantially the same function as the original peptide. The equivalent can be, for example, a fragment of the peptide, a fusion of the peptide with another peptide or carrier, or a fusion of a fragment which additional amino acids.

30

It will of course be understood that a variety of substitutions of amino acids is possible while preserving the structure responsible for activity of the peptide. Conservative substitutions are described in the patent literature, as for example, in United States Patent No 5,264,558 or 5,487,983. It is thus expected, for example,
35 that interchange among non-polar aliphatic neutral amino acids, glycine, alanine,

proline, valine and isoleucine, would be possible. Likewise, substitutions among the polar aliphatic neutral amino acids, serine, threonine, methionine, asparagine and glutamine could be made. Substitutions among the charged acidic amino acids, aspartic acid and glutamic acid, could probably be made, as could
5 substitutions among the charges basic amino acids, lysine and arginine. Substitutions among the aromatic amino acids, including phenylalanine, histidine, tryptophan and tyrosine are also possible. Such substitutions and interchanges are well known to those skilled in the art.

10 Equally, nucleotide sequences encoding a particular product can vary significantly simply due to the degeneracy of the nucleic acid code.

Variants can have a greater or lesser degree of homology as between the variant amino acid/nucleotide sequence and the original.

15

Polynucleotide or polypeptide sequences may be aligned, and percentage of identical nucleotides in a specified region may be determined against another sequence, using computer algorithms that are publicly available. Two exemplary algorithms for aligning and identifying the similarity of polynucleotide sequences
20 are the BLASTN and FASTA algorithms. The similarity of polypeptide sequences may be examined using the BLASTP algorithm. Both the BLASTN and BLASTP software are available on the NCBI anonymous FTP server (<ftp://ncbi.nlm.nih.gov>) under /blast/executables/. The BLASTN algorithm version 2.0.4 [Feb-24-1998], set to the default parameters described in the documentation of variants according
25 to the present invention. The use of the BLAST family of algorithms, including BLASTN and BLASTP, is described at NCBI's website at URL <http://www.ncbi.nlm.nih.gov/BLAST/newblast.html> and in the publication of Altschul, Stephen F., *et al.* (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-
30 34023. The computer algorithm FASTA is available on the Internet at the ftp site <ftp://ftp.virginia.edu/pub/fasta/>. Version 2.0u4, February 1996, set to the default parameters described in the documentation and distributed with the algorithm, is also preferred for use in the determination of variants according to the present invention. The use of the FASTA algorithm is described in W. R.
35 Pearson and D. J. Lipman, "Improved Tools for Biological Sequence Analysis", *Proc.*

Natl. Acad. Sci. USA 85:2444-2448 (1988) and W. R. Pearson, "Rapid and Sensitive Sequence Comparison with FASTP and FASTA, " *Methods in Enzymology* 183:63-98 (1990).

- 5 The following running parameters are preferred for determination of alignments and similarities using BLASTN that contribute to E values (as discussed below) and percentage identity: Unix running command: blastall -p blastn -d embldb -e 10 -G 1 -E 1 -r 2 -v 50 -b 50 -I queryseq -o results; and parameter default values:
- p Program Name [String]
 - 10 -d Database [String]
 - e Expectation value (E) [Real]
 - G Cost to open a gap (zero invokes default behaviour) [Integer]
 - E Cost to extend a cap (zero invokes default behaviour) [Integer]
 - r Reward for a nucleotide match (blastn only) [Integer]
 - 15 -v Number of one-line descriptions (V) [Integer]
 - b Number of alignments to show (B) [Integer]
 - i Query File [File In]
 - o BLAST report Output File [File Out] Optional
- For BLASTP the following running parameters are preferred: blastall -p blastp -d
- 20 swissprotodb -e 10 -G 1 -E 1 -v 50 -b 50 -I queryseq -o results
 - p Program Name [String]
 - d Database [String]
 - e Expectation value (E) [Real]
 - G Cost to open a gap (zero invokes default behaviour) [Integer]
 - 25 -E Cost to extend a cap (zero invokes default behaviour) [Integer]
 - v Number of one-line descriptions (v) [Integer]
 - b Number of alignments to show (b) [Integer]
 - i Query File [File In]
 - o BLAST report Output File [File Out] Optional

30

The "hits" to one or more database sequences by a queried sequence produced by BLASTN, BLASTP, FASTA, or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an

35 overlap over only a fraction of the sequence length of the queried sequence.

DNA sequences from fruiting plants other than *Malus x domestica* which are homologs of *MdPI* may be isolated by high throughput sequencing of cDNA libraries prepared from such plants. Alternatively, oligonucleotide probes based on the sequences for *MdPI* provided in Figure 2 can be synthesized and used to identify positive clones in either cDNA or genomic DNA libraries from other plants by means of hybridization or PCR techniques. Probes should be at least about 10, preferably at least about 15 and most preferably at least about 20 nucleotides in length. Hybridization and PCR techniques suitable for use with such oligonucleotide probes are well known in the art. Positive clones may be analyzed by restriction enzyme digestion, DNA sequencing or the like.

The polynucleotides of the present invention may be generated by synthetic means using techniques well known in the art. Equipment for automated synthesis of oligonucleotides is commercially available from suppliers such as Perkin Elmer/Applied Biosystems Division (Foster City, CA) and may be operated according to the manufacturer's instructions.

The primary importance of identification of the peptide/polynucleotides of the invention is that they enable the reproductive (seed-producing) capacity of fruiting plants to be modulated. This modulation will generally involve a reduction in the functional expression (silencing) of the reproductive peptide.

Any conventional technique for effecting this can be employed. Intervention can occur post-transcriptionally or pre-transcriptionally. Further, intervention can be focused upon the gene itself or on regulatory elements associated with the gene and which have an effect on expression of the encoded peptide. "Regulatory elements" is used here in the widest possible sense and includes other genes which interact with the gene of interest. For example, intervention which targets expression of *MdAP3* peptide is contemplated. *MdAP3* is functionally related to *MdPI* such that down-regulation of *MdAP3* expression will in turn down-regulate *MdPI* (see Jack *et al* (1992) and Goto & Meyerowitz (1994)).

The cDNA and deduced amino acid sequences for *MdAP3* are shown in Figure 6.

Pre-transcription intervention can involve mutation of the gene itself or of its regulatory elements. Such mutations can be point mutations, frameshift mutations, insertion mutations or deletion mutations. These latter mutations include so call "knock-out" mutations in which the gene is entirely ablated.

5

Examples of post-transcription interventions include co-suppression or anti-sense strategies, a dominant negative approach, or techniques which involve ribozymes to digest, or otherwise be lethal to, RNA post-transcription of the target gene.

- 10 Co-suppression can be effected in a manner similar to that discussed, for example, by Napoli *et al* (Plant Cell 2:279-290, 1990) and de Carvalho Niebel *et al* (Plant Cell 7:347-258, 1995). In some cases, it can involve overexpression of the gene of interest through use of a constitutive promoter. It can also involve transformation of a plant with a non-coding region of the gene, such as an intron from the gene or
- 15 5'-non-coding leader sequences.

- Anti-sense strategies involve expression or transcription of DNA with the expression/transcription product being capable of interfering with translation of mRNA transcribed from the target gene. This will normally be through the
- 20 expression/transcription product hybridising to and forming a duplex with the target mRNA.

- The expression/transcription product can be a relatively small molecule and still be capable of disrupting mRNA translation. However, the same result is achieved
- 25 by expressing the target gene in an anti-sense orientation such that the RNA produced by transcription of the anti-sense oriented gene is complementary to all or part of the endogenous target mRNA.

- Anti-sense strategies are described generally by Robinson-Benion *et al.*, (1995),
- 30 Anti-sense techniques, *Methods in Enzymol.* 254(23):363-375 and Kawasaki *et al.*, (1996), *Artific. Organs* 20 (8): 836-848.

- Dominant negative approaches involve the expression of a modified DNA binding/activating protein which includes a DNA binding domain but not a
- 35 activator domain. The result is that the protein binds to DNA as intended but fails

to activate, while at the same time blocking the binding of the DNA binding/activating peptides which normally bind to the same site.

The ribozyme approach to regulation of peptide expression involves inserting
5 appropriate sequences or subsequences (eg. DNA or RNA) in ribozyme constructs (McIntyre CL, Manners JM, *Transgenic Res.* 5(4):257-262, 1996). Ribozymes are synthetic RNA molecules that comprise a hybridizing region complementary to two regions, each of which comprises at least 5 contiguous nucleotides of a mRNA molecule encoded by one of the inventive polynucleotides. Ribozymes possess
10 highly specific endonuclease activity, which autocatalytically cleaves the mRNA.

To give effect to the above strategies, the invention also provides DNA constructs. The constructs include the intended DNA (such as the gene of the invention in anti-sense orientation or a polynucleotide encoding the appropriate DNA binding
15 domain or ribozyme), a promoter sequence and a termination sequence, operably linked to the DNA sequence to be transcribed, which control expression of the gene. The promoter sequence is generally positioned at the 5' end of the DNA sequence to be transcribed, and is employed to initiate transcription of the DNA sequence. Promoter sequences are generally found in the 5' non-coding region of a
20 gene but they may exist in introns (Luehrsen, K.R., *Mol. Gen. Genet.* 225:81-93, 1991) or in the coding region. When the construct includes an open reading frame in a sense orientation (for co-suppression through over-expression) the promoter sequence also initiates translation of the open reading frame. For DNA constructs comprising either an open reading frame in an anti-sense orientation or a non-
25 coding region, the promoter sequence generally consists only of a transcription initiation site having a RNA polymerase binding site.

A variety of promoter sequences which may be usefully employed in the DNA constructs of the present invention are well known in the art. The promoter
30 sequence, and also the termination sequence, may be endogenous to the target *Malus* plant host or may be exogenous, provided the promoter is functional in the target host. For example, the promoter and termination sequences may be from other plant species, plant viruses, bacterial plasmids and the like. Preferably, promoter and termination sequences are those endogenously associated with the
35 reproductive genes.

Factors influencing the choice of promoter include the desired tissue specificity of the construct, and the timing of transcription and translation. For example, constitutive promoters, such as the 35S Cauliflower Mosaic Virus (CaMV 35S) promoter, will affect the activity in all parts of the plant. Use of a tissue specific promoter will result in production of the desired sense or antisense RNA only in the tissue of interest. With DNA constructs employing inducible promoter sequences, the rate of RNA polymerase binding and initiation can be modulated by external stimuli, such as light, heat, anaerobic stress, alteration in nutrient conditions and the like. Temporally regulated promoters can be employed to effect modulation of the rate of RNA polymerase binding and initiation at a specific time during development of a transformed cell. Preferably, the original promoters from the gene in question, or promoters from a specific tissue-targeted gene in the organism to be transformed are used. Other examples of promoters which may be usefully employed in the present invention include, mannopine synthase (mas), octopine synthase (ocs) and those reviewed by Chua *et al.* (Science, 244:174-181, 1989).

The termination sequence, which is located 3' to the DNA sequence to be transcribed, may come from the same gene as the promoter sequence or may be from a different gene. Many termination sequences known in the art may be usefully employed in the present invention, such as the 3' end of the *Agrobacterium tumefaciens* nopaline synthase gene. However, preferred termination sequences are those from the original gene or from the target *Malus* species to be transformed.

The DNA constructs of the present invention may also contain a selection marker that is effective in plant cells, to allow for the detection of transformed cells containing the construct. Such markers, which are well known in the art, typically confer resistance to one or more toxins. One example of such a marker is the NPTII gene whose expression results in resistance to kanamycin or hygromycin, antibiotics which is usually toxic to plant cells at a moderate concentration (Rogers *et al.*, in *Methods for Plant Molecular Biology*, A Weissbach and H Weissbach eds, Academic Press Inc., San Diego, CA (1988)). Alternatively, the presence of the

desired construct in transformed cells can be determined by means of other techniques well known in the art, such as Southern and Western blots.

Techniques for operatively linking the components of the inventive DNA constructs are well known in the art and include the use of synthetic linkers containing one or more restriction endonuclease sites as described, for example, by Maniatis *et al.*, (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbour Laboratories, Cold Spring Harbour, NY, 1989). The DNA construct may be linked to a vector having at least one replication system, for example, *E. coli*, whereby after each manipulation, the resulting construct can be cloned and sequenced and the correctness of the manipulation determined.

The DNA constructs of the present invention may be used to transform a variety of fruiting plants. In a preferred embodiment, the DNA constructs are employed to transform apple and its related species such as pear.

As discussed above, transformation of a fruiting plant with a DNA construct including an open reading frame coding for a peptide encoded by a DNA sequence of the invention wherein the open reading frame is orientated in a sense direction can, in some cases, lead to a decrease in expression of the peptide by co-suppression. Transformation of the plant with a DNA construct comprising an open reading frame in an anti-sense orientation or a non-coding (untranslated) region of a gene will lead to a decrease in the expression of the peptide in the transformed plant.

Techniques for stably incorporating DNA constructs into the genome of target fruiting plants are well known in the art and include *Agrobacterium tumefaciens* mediated introduction, electroporation, protoplast fusion, injection into reproductive organs, injection into immature embryos, high velocity projectile introduction and the like. The choice of technique will depend upon the target plant to be transformed.

Once the cells are transformed, cells having the DNA construct incorporated into their genome may be selected by means of a marker, such as the kanamycin resistance marker discussed above. Transgenic cells may then be cultured in an

before denaturation and transfer to a nitrocellulose filter. Labelled probes may be hybridised to the DNA fragments on the filter and binding determined. DNA for probing may be prepared from RNA preparations from cells. Probing may optionally be done by means of so-called "nucleic acid chips" (see Marshall and
5 Hodgson (1998)).

The invention will now be illustrated with reference to the following non-limiting experiments.

10 **EXPERIMENTAL**

Methods and Materials

Cloning MdPI using PCR approaches

15 Total RNA was isolated from 'Granny Smith' apple flowers using the method described by Chang *et al* (1993). Poly(A) mRNA was purified from the total RNA using the mRNA Purification Kit (Pharmacia, Sweden). cDNA was synthesized from the mRNA using the ZAP cDNA Synthesis Kit (Stratagene, CA, USA). DNA fragments were amplified from templates of cDNA using two degenerative PCR
20 primers P1 CGGAATTCATGGGNGMGNGGNAARRT-3' and P2 CGCTCGAGGATCCGGYTGNATNGGYTGNAC-3' (N=ATGC, M=AC, R=AG, Y=CT). The primers were designed according the conserved amino acid sequences MGRGKI in the MADS-box domain and VQPM/IQP in the C-terminal region (Fig. 2) in an alignment of PI, GLOBSA, FBP3, SLM2 and pMADS2. The underlined Eco RI and
25 Bam HI sites were used for cloning the PCR products. The PCR amplification conditions were as follows: initial denaturation at 94°C for 4 min; then 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 3 min, and with a final extension of 5 min at 72°C. Several bands were detected from the PCR on agarose gels and DNA in a band of the expected size (630 bp) was cloned into Bluescript SK (Stratagene,
30 CA, USA) following Eco RI and Bam HI digestion. After the sequences of cloned fragments were determined, two nested PCR primers, P3 and P4 (Fig. 2) were designed using the sequences within the K-box and were used to amplify the 3' region of MdPI cDNA together with a 3' RACE primer GAGAGAGAACTAGTCTCGAG-3'. The PCR conditions were the same as above except for the anneal temperature

reduced to 50°C. The amplified fragments were cloned into pGEM-T EASY Vector (Promega).

5 Genomic fragments of MdPI were amplified using primers P5 and P6, P3 and P7 (Fig. 2). PCR conditions were: initial denaturation at 94°C for 2 min; then 10 cycles of 94°C for 15 sec, 58°C for 30 sec; and 20 cycles of 94°C for 15 sec, 58°C for 30 sec and 68°C for 5 min plus cycle elongation of 20 sec for each cycle; and with a final extension of 5 min at 86°C. The amplified fragments were cloned into pGEM-T EASY Vector. Expand High Fidelity PCR System (Boehringer Mannheim) was used
10 for all PCR experiments.

DNA sequence determination

Nucleotide sequences of MdPI clones were determined using the automatic sequencer ABI PRISM model 377(CA, USA) with universal forward and reverse
15 primers. To obtain complete sequences, gene specific primers were designed and ordered from BRL Life Technologies.

Northern and Southern analysis using MdPI on apple tissues

Total RNA was isolated as described by Chang *et al* (1993) from 'Granny Smith' and
20 Rae Ime apple tissues. Northern blots were prepared as described by Dong *et al* (1997). The northern blot contained RNA isolated from expanding leaves, unopened flowers, and fruit at 2 days and 1, 4 and 8 weeks following hand-pollination. At 4 weeks after pollination, apple fruit is large enough to allow for easy separation into the three main tissue types namely; core, cortex and skin.

25

DNA was isolated from leaf tissue of Granny Smith and Rae Ime using the method of Rogers and Bendich (1988). Southern blots were prepared by digesting apple DNA (approximately 20 µg per lane) with EcoRI or HindIII, separating DNA fragments on 0.7% agarose gel and transferring them to Hybond-N+ membrane.

30

Northern and Southern blots were probed with 32P-dCTP labelled PI cDNA clone lacking the MADS-box sequence to significantly reduce cross hybridization 32P-dCTP labelled MADS-box DNA fragments. The blots were hybridized in 0.5M NaPO4 buffer (pH 7.2) with 1 mM EDTA and 7% SDS at 65°C and washed using 0.4x SSC

and 0.2% SDS at 65°C. Hybridisation signals were detected using a Storm 840 PhosphorImager (Molecular Dynamics, Sunnyvale, California, USA).

Results/Discussion

5

Flowers of the majority of apple taxa bear 5 sepals, 5 petals, 9-20 stamens (Fig. 1a) and an inferior ovary. These flowers develop into a pome fruit that consists of fleshy cortex tissue derived from the fused bases of sepals, petals and stamens, and the core tissue derived from fertilised ovary containing 5 carpels and up to 10 seeds (Pratt, 1988) (Fig. 1b). In contrast, flowers of Rae Ime show no petal or stamens but increased numbers of styles (Fig. 1c). These flowers develop into seedless fruit without the need for pollination. These seedless fruit have two whorls of carpels, five carpels in the lower whorl and 9 to 10 in the upper whorl (Fig. 1d). The fruit also has duplicated whorls of calyxes (Fig. 1e) that are the remains of sepals, compared to one calyx whorl in a normal apple (Fig. 1f). The mature seedless fruit are close to normal apple fruit size, but the fruit cores are relatively smaller (Fig. 1g).

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15

Several apple varieties, such as Spencer Seedless and Wellington Bloomless (Tobutt, 1994), have been described with a very similar flower and fruit structure to that of Rae Ime. Anatomy studies of the vascular connections show that the upper whorl of carpels has been transformed from the stamens and the second whorl of sepals from petals (Brase, 1937). In the *Arabidopsis pi* and *ap3* mutants, flowers have no petals or stamens but have double the number of sepals and carpels (Goto and Meyerowitz, 1994; Jack *et al.*, 1992).

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25

A difference between Rae Ime apple and *pi Arabidopsis* is that the former produces parthenocarpic fruit but the latter does not. Up to 6 apple varieties have been recorded to produce apetalous flowers and parthenocarpic fruit in different countries. Many of these records can be traced back to several centuries ago (Brase, 1937; Tobutt, 1994). This indicates some of the apple mutants may have occurred independently.

30

Genetic analysis has been performed using two apetalous/parthenocarpic varieties, Spencer Seedless and Wellington Bloomless. Crossing pollen from the

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to intron 6, are highly conserved compared to the positions of 5 introns in *PI* gene (Fig. 2). We conclude that *MdPI* is the *PI* homolog based on these results having highest sequence identity and conserved intron positions and mRNA expression patterns.

5

In an experiment to examine whether there is a mutation in *MdPI* of Rae Ime, the expression level of *MdPI* in flower buds was determined. Expression of *MdPI* in the apetalous Rae Ime flower buds is not detected, but is readily detected in normal flower buds of the Granny Smith variety (Fig. 3). In *Arabidopsis pi* mutants, *PI* expression is reduced or abolished in flower buds (Goto and Meyerowitz, 1994).

10

A second experiment compared RFLP patterns for Rae Ime with normal apple cultivars using the *MdPI* cDNA as a probe. Southern hybridisation shows different RFLP patterns between Rae Ime and Granny Smith with both *EcoRI* and *HindIII* digestion (Fig. 4) although Granny Smith RFLP pattern is conserved in another apple variety Royal Gala (data not shown). Both the expression and RFLP data indicate that the *MdPI* gene in Rae Ime has been mutated. As both enzyme digestions reveal RFLP differences, the mutation is likely to be a gross change in gene structure rather than a point mutation

20

Genomic DNA fragments were cloned from Granny Smith and Rae Ime using two primers P3 and P7 designed with *MdPI* cDNA sequence. The Rae Ime fragments were 11 kb while the Granny Smith fragments were 2 kb (Fig. 5a). These fragments show a hybridisation signal to the *MdPI* cDNA probe (Fig. 5b). Clones containing these fragments were partially sequenced from two ends. The Rae Ime fragments have the same sequence to the Granny Smith fragments at two ends, but with an insertion in the intron 4 of *MdPI* gene in Rae Ime (Fig. 5b). The insertion sequences were found to be an LTR retrotransposon. This result confirmed that there is a mutation in the *MdPI* gene in Rae Ime.

30

By way of confirmation that it is the mutation of the *MdPI* gene which is responsible for the parthenocarpic phenotype, the *MdPI* gene from two further parthenocarpic apple varieties, Spencer Seedless and Wellington Bloomless, was sequenced (data not shown). This revealed an approximately 9 kb insertion in each gene. Thus, in the three parthenocarpic apple varieties examined, there are

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two different insertion sites in the *MdPI* gene both of which lead to the parthenocarpic phenotype. Spencer Seedless and Wellington Bloomless have the same insertion site, which is different from that in Rae Ime (Fig. 5c). These confirmatory results demonstrate that independent mutations in *MdPI* generate the same apetalous/parthenocarpic phenotype.

The difference in fruit development between Rae Ime apple and *pi Arabidopsis* may be explained in two different ways. Firstly, *MdPI* may have different function compared to *PI* in influencing ovary and fruit development. Sufficient functional differences have been shown for homologs of floral homeotic genes in different plant species (Causier *et al.*, 1999). Secondly, apple fruit develops from both ovary and the fused bases of sepals, petals and stamens (Pratt, 1988). Apple differs from tomato and *Arabidopsis*, two model systems often used in studies of fruit development, where the fruit or silique develops from ovary tissue only (Weigel and Mererowitz, 1994; Gillaspay *et al.*, 1993). The differences in fruit structure may cause different fruit development after a mutation in a floral homeotic gene.

INDUSTRIAL APPLICATION

In its primary aspect, the invention has application in modulating, and in particular reducing or eliminating seed-bearing capacity in fruiting plants. Such plants have utility in horticulture.

It will also be possible to employ the polynucleotides of the invention in breeding programmes to monitor the progress made towards breeding a stable seedless fruiting plant.

The availability of reproductively null or sterile trees has the additional advantage that it will be possible to introduce further exogenous genetic material into those trees without the risk that the material will be passed on to other trees.

Those persons skilled in the art will appreciate that the specific description provided is exemplary only, and that modifications and variations may be made without departing from the scope of the invention.

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CLAIMS:

1. A fruiting plant which has been genetically modified such that it does not functionally express:
 - (i) a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and/or
 - (ii) a peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof,which plant produces seedless or sterile fruit.
2. A fruiting plant which contains a polynucleotide encoding a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof and in which the functional expression of said peptide within said plant has been disrupted such that the plant produces seedless or sterile fruit.
3. A fruiting plant according to claim 1 or claim 2 which produces a pome fruit.
4. A fruiting plant which contains:
 - (a) a polynucleotide encoding a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and
 - (b) a polynucleotide encoding a peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof,and in which the functional expression of said peptide encoded by polynucleotide (a) within said plant has been disrupted such that the plant produces seedless or sterile fruit.
5. A plant as claimed in claim 4 wherein functional expression of said peptide encoded by polynucleotide (a) is disrupted directly.
6. A plant as claimed in claim 4 wherein functional expression of said peptide encoded by polynucleotide (a) is disrupted indirectly.

7. A plant as claimed in claim 6 wherein said indirect disruption is effected through disrupting functional expression of the peptide encoded by said polynucleotide (b).
8. A plant as claimed in any one of claims 4 to 7 wherein said plant is one which produces pome fruit.
9. A plant as claimed in claim 8 wherein said polynucleotide (a) has the coding sequence of SEQ ID NO: 1.
10. A plant as claimed in claim 8 wherein said polynucleotide (a) has the nucleotide sequence of SEQ ID NO: 1.
11. A plant as claimed in claim 8, claim 9 or claim 10 in which said polynucleotide (b) has the coding sequence of SEQ ID NO: 3.
12. A plant as claimed in claim 8, claim 9 or claim 10 wherein said polynucleotide (b) has the nucleotide sequence of SEQ ID NO: 3.
13. A polynucleotide which encodes a peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof.
14. A polynucleotide as claimed in claim 13 which comprises the coding sequence of SEQ ID NO: 1.
15. A polynucleotide as claimed in claim 13 which comprises the nucleotide sequence of SEQ ID NO: 1.
16. A polynucleotide which encodes a peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof.
17. A polynucleotide as claimed in claim 16 which comprises the coding sequence of SEQ ID NO: 3.
18. A polynucleotide as claimed in claim 16 which comprises the nucleotide sequence of SEQ ID NO: 3.
19. A DNA construct which includes a polynucleotide as claimed in any one of claims 13 to 18.

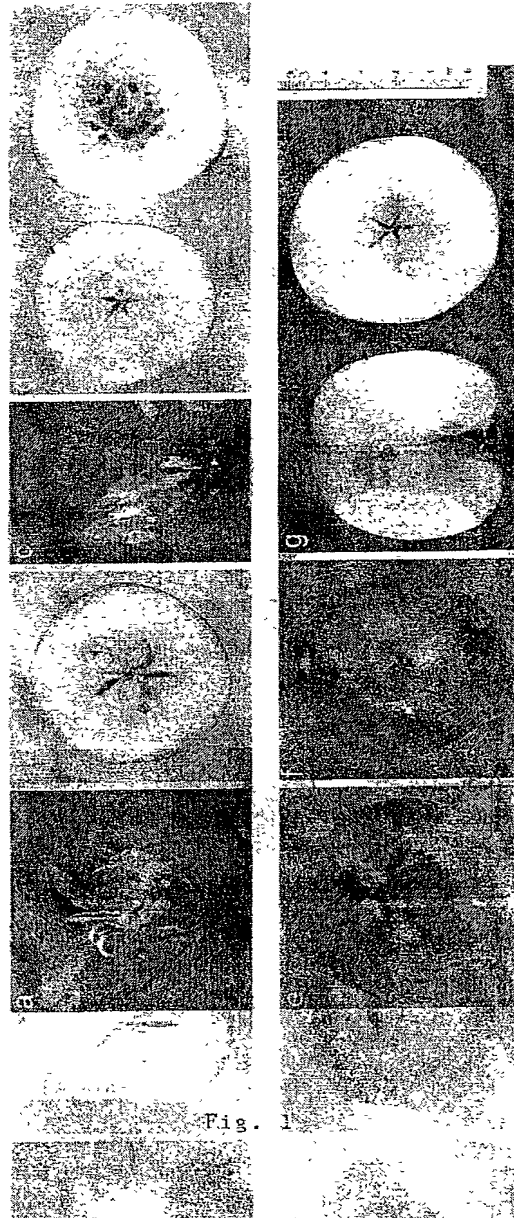
20. A DNA construct comprising, in the 5'-3' direction:
- (a) a promoter sequence;
 - (b) an open reading frame polynucleotide as defined in any one of claims 13 to 18; and
 - (c) a termination sequence.
21. A DNA construct as claimed in claim 20 wherein the open reading frame polynucleotide is in a sense orientation.
22. A DNA construct as claimed in claim 20 in which the open reading frame polynucleotide is in an anti-sense orientation.
23. A DNA construct comprising, in the 5'-3' direction:
- (a) a promoter sequence;
 - (b) a non-coding region of a gene coding for the peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and
 - (c) a termination sequence.
24. A DNA construct comprising, in the 5'-3' direction:
- (a) a promoter sequence;
 - (b) a non-coding region of a gene coding for the peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof; and
 - (c) a termination sequence.
25. A DNA construct as claimed in claim 23 or claim 24 in which the non-coding region is in a sense orientation.
26. A DNA construct as claimed in claim 23 or claim 24 in which the non-coding region is in an anti-sense orientation.

27. A DNA construct comprising, in the 5'-3' direction:
- (a) a promoter sequence;
 - (b) a polynucleotide comprising a nucleotide sequence complementary to at least part of a sequence coding for the peptide having the *MdPI* amino acid sequence of SEQ ID NO: 2 or a functionally equivalent variant thereof; and
 - (c) a termination sequence.
28. A DNA construct comprising, in the 5'-3' direction:
- (a) a promoter sequence;
 - (b) a polynucleotide comprising a nucleotide sequence complementary to at least part of a sequence coding for the peptide having the *MdAP3* amino acid sequence of SEQ ID NO: 4 or a functionally equivalent variant thereof; and
 - (c) a termination sequence.
29. A transgenic cell of a fruiting plant which includes a DNA construct as claimed in any one of claims 19 to 28.
30. A transgenic cell as claimed in claim 29 in which said fruiting plant is one which produces a pome fruit.
31. A fruiting plant containing a transgenic cell as claimed in claim 29.
32. A fruiting plant containing a transgenic cell as claimed in claim 30.
33. A seedless or sterile fruit which is produced by a fruiting plant as claimed in any one of claims 1, 2, 4-7 and 31.
34. A seedless or sterile pome fruit which is produced by a fruiting plant as claimed in any one of claims 3, 8 to 12 and 32.

Abstract

The invention provides fruiting plants that produce seedless or sterile fruit. The production of seedless or sterile fruit is the result of genetic modification which prevents or disrupts functional expression of the MdPI peptide of SEQ ID NO: 2 or a variant thereof, or of the MdAP3 peptide of SEQ-ID NO: 4 or a variant thereof, or both.

1/4



2/4

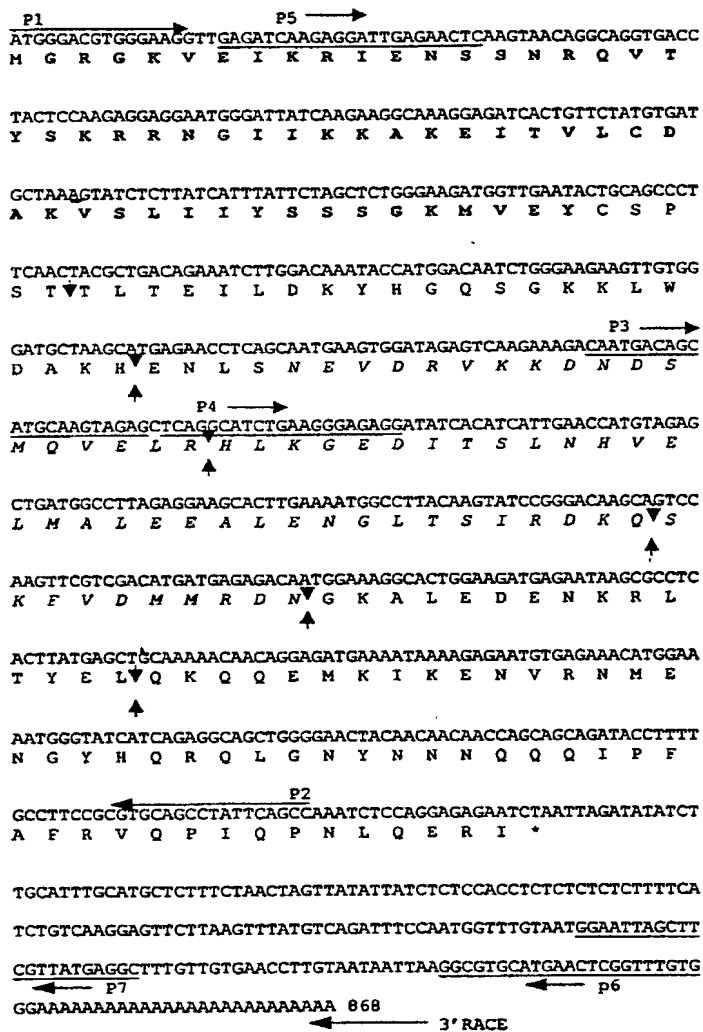


Fig. 2

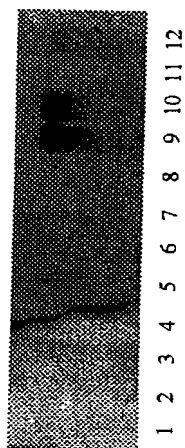


Fig. 3

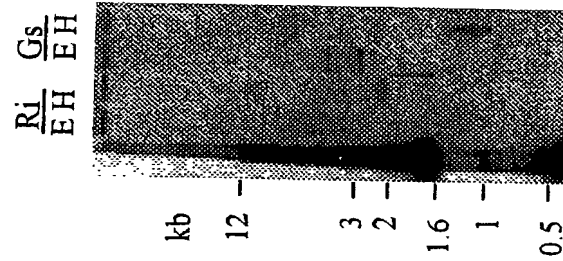
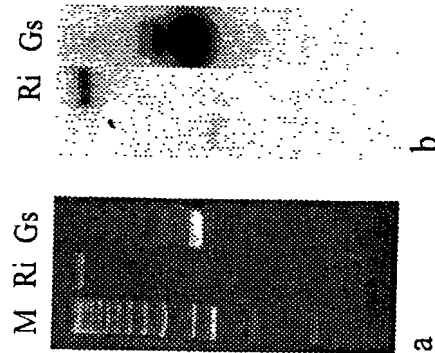


Fig. 4



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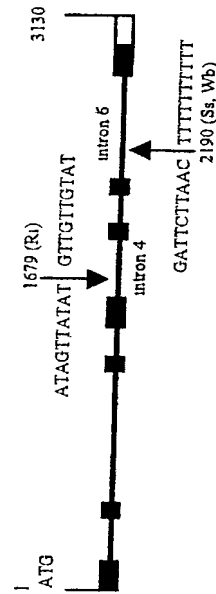


Fig. 5

4/4

ATGGCGCGCGGGAAGATTGAAATCAAGCTGATCGAAAACAGACCAACAGGCAGGTGACC
M A R G K I E I K L I E N Q T N R Q V T
TACTCCAAGAGAAGAAATGGGATCTTCAAGAAGGCTCAGGAGCTCACCGTTCTCTGTGAT
Y S K R R N G I F K K A Q E L T V L C D
GCCAAGGTCTCCCTCATTATGCTCTCCAACACTAATAAAATGCACGAGTATATCAGCCCT
A K V S L I M L S N T N K M H E Y I S P
ACCACTACGACCAAGAGTATGTATGATGACTATCAGAAAATATGGGGATCGATCTGTGG
T T T T K S M Y D D Y Q K T M G I D L W
AGGACACACGAGGAGTCGATGAAAGACACCTTGTGGAAGTTGAAAGAGATCAACAATAAG
R T H E E S M K D T L W K L K E I N N K
CTGAGGAGAGAGATCAGGCAGAGGTTGGGCCATGATCTAAATGGCCTGAGCTTTGACGAG
L R R E I R Q R L G H D L N G L S F D E
CTGGCTTCTCTTGACGATGAGATGCAGTCTTCCTTGGATGCCATACGTCAAAGGAAGTAC
L A S L D D E M Q S S L D A I R Q R K Y
CATGTGATCAAACTCAGACGGAGACCACCAAGAAGAAGGTTAAGAACTTGGAGCAAAGA
H V I K T Q T E T T K K K V K N L E Q R
AGAGGAAACATGCTGCATGGCTATTTTGACCAGGAAGCAGCCGGCGAGGATCCACAGTAT
R G N M L H G Y F D Q E A A G E D P Q Y
GGTTATGAGGACAATGAGGGAGACTACGAATCTGCACTTGCAATTGTCAAATGGGGCGAAT
G Y E D N E G D Y E S A L A L S N G A N
AACTTGACACTTTCCACCTCCACCACCCTAACCTCCACCACGGAGGAAGCTCGCTCGGC
N L Y T F H L H H P N L H H G G S S L G
TCCTCCATTACTCATCTGCACGATCTCCGCCTTGCTTGATCGTGATCTGAGATATGATTA
S S I T H L H D L R L A *
ATCATCACTAAGTTATATATTAAGGTCACCTATAACTGCTTTTGCTCTAAAGTGTTTGCT
TGGTGACTATCTTTAGGCAAGGAGTTAGACTTGGACTACCTCTGAAAACAGATGCATAAA
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ATAAAAAAAAAAAAAAAAAAAAA 982

Fig. 6

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ALL PATENTS, INCLUDING DESIGN
FOR APPLICATION BASED ON PCT; PARIS CONVENTION;
NON PRIORITY, OR PROVISIONAL APPLICATIONS

As a below named inventor, I declare that my residence, post office address and citizenship are stated below next to my name, the information given herein is true, that I believe that I am the original, first and sole inventor (if only one name is listed at 201 below), or an original, first and joint inventor (if plural inventors are named below at 201-203, or on additional sheets attached hereto) of the subject matter which is claimed and for which patent is sought on the invention entitled.

SEEDLESS FRUIT PRODUCTION

which is described and claimed in: ☒ PCT International Application No **PCT/NZ00/00176** filed **September 7, 2000**
☐ the attached specification ☒ the specification in application Serial No _____ filed **March 6, 2002**
(if applicable) and amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed

Prior Foreign Application(s)

337688 **NEW ZEALAND** **7 September 1999**
(Number) (Country) (Day/Month/Year Filed)

Priority Claimed

☒ Yes ☐ No

(Number) (Country) (Day/Month/Year Filed)

☐ Yes ☐ No

(Number) (Country) (Day/Month/Year Filed)

☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below

Application No. _____ Filing Date _____ Application No. _____ Filing Date _____

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)

(Filing Date)

(Status: patented, pending, abandoned)

9- POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys (Registration No.) to prosecute this application, receive and act on instructions from my agent, and transact all business in the Patent and Trademark Office connected therewith: **HARVEY B. JACOBSON, JR. (20,851); JOHN CLARKE HOLMAN (22,769); MARVIN R. STERN (20,640); ALLEN S. MELSER (27,215); MICHAEL R. SLOBASKY (26,421); JONATHAN L. SCHERER (29,851); IRWIN M. AISENBERG (19,007); WILLIAM E. PLAYER (31,409); YOON S. HAM (45,307) and NATHANIEL A. HUMPHRIES (22,772).**

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*Inventor(s) name must include at least one unabbreviated first or middle name

1-00 201	FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY
2-00 202	FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY
203	FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME
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SIGNATURE OF INVENTOR 201*	SIGNATURE OF INVENTOR 202*	SIGNATURE OF INVENTOR 203*
DATE	DATE	DATE

☐ Additional inventors are named on separately numbered sheets attached hereto.

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